

CHANGES TO THE SPECIFICATION

Please substitute the following marked up paragraph(s) for the paragraph(s) now appearing in the currently filed specification:

Please replace the following paragraphs:

On page 3, lines 19-20.

WO 04297 (Karger et al) and SE 0004594-8-WO 0247913 (Gyros AB) suggest to have microchannel structures in radial or spoke arrangement.

On page 3, lines 21-29.

A number of publications referring to the use of centrifugal force for moving liquids within microfluidic systems have appeared during the last years. See for instance WO 9721090 (Gamera Bioscience), WO 9807019 (Gamera Bioscience) WO 9853311 (Gamera Bioscience), WO 9955827 (Gyros AB), WO 9958245 (Gyros AB), WO 0025921 (Gyros AB), WO 0040750 (Gyros AB), WO 0056808 (Gyros AB), WO 0062042 (Gyros AB) and WO 0102737 (Gyros AB) as well as ~~unpublished items PCT/EP00/12478~~ WO 0147637 (Gyros AB), ~~PCT/EP01/00653~~ WO 0154810 (Gyros AB), ~~PCT/EP00/130244~~ WO 0147638 (Gyros AB), ~~PCT/EP00/13145~~ and WO 0146465.

On page 12, lines 6-16.

Between parts having different functions there may be valves that can be overcome by increasing the force driving the liquid. For variants utilizing spinning, this may for instance be accomplished by increasing the spinning and/or utilizing pressure built up within the structure due to addition of a new portion of liquid combined with spinning. See for instance WO 0040750 (Gyros AB) and ~~PCT/EP00/13145~~ WO 0146465 (Gyros AB). Valves may be based on capillary junctions (WO 9807019 (Gamera Bioscience)) or hydrophobic breaks (WO 9958245 (Gyros AB) or on thermic properties of the valve material. The latter kind of

valves may be illustrated by so called sacrificing valves (WO 9853311 (Gamera Bioscience)) for instance containing a plug of wax-like material, or reversible valves, for instance containing a thermoreversible polymer in the form of a plug (WO 0102737 (Gyros AB)).

Page 15, lines 20-31.

Figure 2 illustrates another variant of a suitable microchannel structure. It has two inlet ports (5,6) that may be used for application of sample, washing liquids and desorption liquid. One of the inlet ports (5) is connected to an application area/channel (7) that may be common to several microchannel structures in the same device. This first inlet port (5) is connected to one of the shanks (8) in a U-shaped channel part via the application area/channel (7). The other inlet port (6) is connected to the other shank of the U. In the lower part of the U there is an exit conduit (9) leading to an MS-port (10). In the channel (11) between the exit conduit (9) from the U and the MS-port (10) there may be a zone (12) containing a separation medium. From the MS-port (10) there may be a waste channel (13) leading to a waste space (14) that may be common for several microchannel structures in the same device. There may be a valve function, for instance in the form of a hydrophobic break, in the exit conduit (9).

Page 16, lines 1-21.

Figure 3 illustrates another alternative of a microchannel structure which comprises a separate sample inlet port (14), an MS-port (15) and therebetween a microchannel structure that may be used for sample preparation. In this variant there is a volume-defining unit (16) between the two ports (14,15) with an over-flow conduit (17). At the lower part of the volume-defining unit (16) there is a first exit conduit (18) leading to one of the shanks (19) of a U-shaped channel part. The other shank (20) of this U may be connected to an inlet port (21) for washing and desorption liquids. At the lower part of the U-shaped channel part there may be a second exit conduit (22) leading into one of the shanks (23) of a second U-shaped channel part. The other shank (24) may be connected to a waste channel (25b) that after a bend (26) may end in a waste chamber (25a). At the lower part of this second U-formed channel part there may be a third exit conduit (27) leading into the MS-port (15) that may contain an EDI area or an electrospray unit. In order to control the flow in the structure, valve functions are preferably located in the first exit conduit (18), for instance immediately downstream the volume-defining unit (16), possibly also in the second exit conduit (22), for instance immediately after the first U-shape, and in the third exit conduit (27), for instance

immediately after the second U-shaped channel part. The valves may be of the types discussed above with preference for hydrophobic breaks. A suitable adsorbent (28) as discussed above may be placed in the shank (23) of the second U-shaped channel partsecond exit conduit (23) and may also function as a valve. In case the adsorbent is in the form of particles they are preferably kept in place by a constriction of the inner walls of the conduits.

Page 16, lines 23-32 through page 17, lines 1-15.

The structure presented in **figure 3** is adapted for transporting the liquid with centrifugal forces, i.c. with the structure present in a disc and oriented radially outwards from the centre of the disc. At start the volume-defining unit (16) is filled up somewhat above the over-flow channel (17). By overcoming a valve function located in the first exit conduit (18), the liquid will pass into the first U-shaped channel part and down through the adsorbent where the analytes are captured. The remaining liquid containing non-analyte components will pass out into the waste channel (25b). In the next step, washing liquids may be applied through the inlet port (21), i.e. through the second shank (20) of the first U-shaped channel part or via the same inlet port (14) as the sample. Also these liquids will pass out into the waste channel (25b). Subsequently, a desorption liquid is applied through either of the two inlet port (14,21) and allowed to pass through the valve function in the third exit conduit (27). The desorption liquid containing released analyte or analyte-derived entities is passed downstream, for instance into the MS-port (15). The operations are preferably carried out while spinning the disc. If the valves are in the form of hydrophobic breaks they can be passed by properly adapting the g-forces, i.c. by the spinning. By properly balancing the hydrophilicity/hydrophobicity of a liquid, passage or non-passage through a valve may be controlled without changing the spinning speed. This is illustrated by utilizing a hydrophobic break as the valve in the third exit conduit (27) combined with utilizing water-solutions as samples and as washing liquids and liquids containing organic solvents as desorption liquids. In the alternative, valves that are opened by external means can be used. By placing the outlet to the first exit conduit (18) at a distance above the lowest part of the volume-defining unit (16) particulate matters, if present in the sample, will sediment and be retained in the volume-defining unit (16) when it is emptied through the first exit conduit (18).

Page 17, lines 27-29.

These kind of flow systems has been described in WO 0040750 (Gyros AB) and PCT/EP00/13145-WO 0146465 (Gyros AB) which are hereby incorporated by reference.

Page 18, lines 30-32 through page 19 lines 1-15.

Figure 4 illustrates an MS-port suitable for electrospray ionisation in a mass spectrometer. This kind of port may be located where an MS-port has been indicated in any of the structures given in **figures 1-3**. The MS-analyte may thus be collected in an MS-port comprising a collection zone (30), which zone is in fluid communication via the electrospray conduit (31) with the outlet orifice (32). The electrospray conduit may be in the form of a tip. The MS-analyte is entering via conduit (32). As indicated in **figure 4**, orifices Θ of the electrospray tip are preferably positioned on the edge of a disc. Typical disc-forms have been discussed above. In use an electrospray orifice is matched to the sampling orifice of a mass spectrometer and liquid in the electrospray zone--tip (31) is sprayed into the mass spectrometer. In a preferred variant the disc is circular. The arrangement of the electrospray tips is preferably annular around the axis for spinning during preparation of the sample. The tips are preferably located in the edge of the disc and provide a radial spray direction. In the alternative the annular arrangement may be in a flat side of the disc and with a spray direction out of the plane of the side and preferably also with a component that is directed radially. Annular arrangements preferably in the edge of a circular disc will simplify accurate positioning of the electrospray direction relative to the sample application opening of a mass spectrometer.

Page 19, lines 26-32 through page 20, lines 1-5.

A liquid solution suitable for ESI MS analysis consist of an organic solvent:water mixture including a lower concentration of acid or base. The composition of the solvent is important especially with regard to surface tension and conductivity. A low surface tension and conductivity is desirable as-to obtain an efficient desolvation and ionization process as well as a stable spray. If the sample is dissolved in water only, a so-called make-up solvent is preferably added (external delivery) as-to aid in the above mentioned process. A make-up solvent is typically configured co-axially (sheat-flow) around the nanospray tip. A make-up

gas (typical N₂) is also sometimes added (e.g., co-axially) as to aid in the desolvation process. Creation of a suitable liquid composition of the MS-sample may also be part of the sample preparation process taking place upstream the MS port in other parts of a microchannel structure.

Page 20, lines 24-29.

Typical electrospray nozzles are available from a number of manufacturers, for instance New Objective, MA, U.S.A. A variant that is believed to have advantages for microfluidic devices is presented in SE-0004594-8 WO 0247913 (Gyros AB). See also WO 9704297 (Karger et al), US 5,969,353 (Hsieh) and US 6,110,343 (Ramsey et al) discussed above.

Page 22, lines 29-32 through page 23, lines 1-11.

Figure 5a shows a common conducting layer (54) at the bottom of the device which layer encompasses layer (I) (53) of each EDI area (52). A non-conducting layer (II) (55) is placed between layer (I) (53) and the EDI surface (51). Figure 5b shows a variant, which is similar to the variant in figure 5a, but the common conducting layer is embedded within the material from which the device is fabricated. Layer (II) (55) is present. In figure 5c there is a common conducting layer (54) comprising the upper surfaces of the EDI areas. In figure 5d there is no common conducting layer. Layer (I) is in the upper surfaces of the EDI areas. The different layers (I) (~~54~~53) are isolated from each other. Figures 5e shows a variant in which there is a continuous conducting layer (54) above layer (I) (53) of the EDI areas. This conducting layer (54) has openings (56) corresponding to the openings of each MS-port and may be the surface of the upper or lower side of a lid covering the microchannel structure (the lid is not shown). Figure 5f shows a variant in which there is a common continuous conductive layer comprising layer (I). Layer (I) corresponds to the EDI surfaces. The continuous layer also encompasses the walls of the depressions in the EDI ports.

Page 23, lines 22-30.

Figures 7a-b illustrate an EDI MS-port which the opening to the EDI surface is defined by a hole (39) in a lid (40) which in this case is transparent. One can see the incoming microchannel (41), which opens to a circular depression (42) with a diameter, which is less than the diameter of the hole (39). Layer (I) (43), EDI area (44, between the dotted lines),

EDI surface (4445) are shown. The conductive layer extends from layer (I). This design in which the MS-port provides an opening which is greater than the EDI area will facilitate for an incident beam to cover any spot of the EDI surface.

Page 25, lines 31-32 through page 26, lines 1-8.

At least a part of the microchannel structure may have a surface that has been derivatised and/or hydrophilized, for instance by being coated with a non-ionic hydrophilic polymer according to the principles outlined in International Patent Application Publication (PCT/EP00/12478 WO 0147637 (Gyros AB)). This also includes functionalisation in order to introduce one or more structures that are capable of interacting with the sample analyte or with one or more of the reagents added. Surfaces may be made of copper, gold, platinum, stainless less etc, for instance by metallization, in order to enable a desired derivatization or for providing a conducting surface, for instance in an MS-port. Gold surfaces for instance may be derivatized by reaction with thiol-containing compounds that have a desired functionality, for instance hydrophilicity.